Effect of Some Analgesic and Anti-Inflammatory Agents on Sodium Pentobarbital Anesthesia. (24065)

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(Introduced by W. M. Govier)
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It is commonly supposed that combinations of barbiturates with low-potency analgesics clinically show some type of "synergism" in relief of pain or production of increased hypnosis. In this communication we wish to report the effect of analgesics and anti-inflammatory drugs on brain barbiturate concentration under anesthesia, in order to gain insight into the mechanism of the increased hypnosis mentioned above. Duration of hypnosis was assessed concomitantly.

Methods. 1. *Prolongation of pentobarbital sleeping time.* Carworth male mice ranging in weight from 20-24 g were fasted for a period of 18 hours and distributed into 6 groups of 10 animals each. The compound under test was administered orally to 3 of these groups. One hour later, these groups received logarithmically spaced doses of sodium pentobarbital intraperitoneally. The same doses of sodium pentobarbital were administered to the remaining 3 groups of mice, previously untreated, which served as controls. Barbiturate-induced hypnosis was measured as a function of the duration of loss of the righting reflex (sometimes referred to as "sleeping time"). Relative potencies of pentobarbital with and without adjuvants were obtained by the method of Finney(1).

2. *Brain pentobarbital determinations.* Sodium pentobarbital levels in mouse brains were determined by the procedure of Brodie *et al.*(2) with some modifications: Carworth Farms male mice weighing 17-24 g were fasted overnight. Compounds tested were administered orally one hour prior to intraperitoneal administration of sodium pentobarbital (65 mg/kg). Two hours after sodium pentobarbital administration, the animals were killed by cervical dislocation and the brains removed immediately. Four mouse brains, having a total weight of approximately 1.7 g were homogenized in 4 ml distilled water. The homogenate was placed in a glass stoppered extraction tube to which was added 1.5 g sodium chloride, 1.0 ml buffer pH 5.5, and 60 ml petroleum ether containing 1.5% isoamyl alcohol. The petroleum ether-isoamyl alcohol solution was added in the cold room. The tubes were shaken in an Eberbach shaker (approximately 100 oscillations/min, 4 cm path), in a cold room at 4°C. At the end of 1 hour, a 40 ml aliquot of the petroleum ether phase was removed and shaken with 4 ml buffer pH 11 (4.25 ml 10 *N* NaOH + 200 ml Na_2HPO_4) for 3 minutes at room temperature. The petroleum ether phase was withdrawn and the aqueous phase transferred to a centrifuge tube. It was necessary to centrifuge in order to separate the aqueous from the petroleum ether phase completely. The aqueous phase was then read in Beckman DU Spectrophotometer at 240 mμ. Standards were prepared by taking 1 ml of various dilutions of a sodium pentobarbital solution and adding these to 5 ml buffer pH 11. Optical densities obtained with these dilutions were equivalent to the standard values obtained when sodium pentobarbital was carried through the extraction procedure. Sodium pentobarbital added to brain homogenate at concentrations of 10-100 μg showed a mean recovery of 93-99%. The brains of untreated mice were assayed simultaneously with those of mice receiving compounds but no sodium pentobarbital. In order to allow for day to day variations in response of the animals to sodium pentobarbital, controls receiving sodium pentobarbital alone were run with each experimental group. Brain blanks varied from 1.9-5.6 μg/g of brain.

Results. Significant prolongation of barbiturate hypnosis was produced by previous administration of aminopyrine or phenylbutazone at 25 mg/kg (Table I). Prednisone (25 mg/kg), morphine (1 and 25 mg/kg, or acetylsalicylic acid (25 or 250 mg/kg) which were also tested but not listed in Table I did

TABLE I. Effect of Aminopyrine and Phenylbutazone on Duration of Barbiturate Hypnosis.

Agents	Dose (mg/kg)	No. anesthetized*	Duration (min.)	Potency (90% confidence limits)
Na pentobarbital	65	10	162.4	1.
	48	10	75.6	
	35	8	13.7	
Idem + aminopyrine (25 mg/kg)	65	10	240.0	1.34 (1.21–1.49)
	48	10	153.0	$p < .001$
	35	7	108.0	
Na pentobarbital	65	10	106.0	1.
	48	10	79.6	
	35	7	9.0	
Idem + phenylbutazone (25 mg/kg)	65	10	232.6	1.28 (1.15–1.46)
	48	10	104.6	$p < .001$
	35	10	25.1	

* No. anesthetized from groups of 10 mice.

TABLE II. Effect of Several Agents on Brain Na Pentobarbital Concentration (μg/g Brain).

	Statistical analyses*					
	$\bar{x}$		S.E.†		N	P
Compound (mg/kg)	Test	Control	Test	Control		
Phenylbutazone (25)	20.7	14.3	1.5	1.5	18	<0.01
Aspirin (250)	13.2	11.9	1.0	1.0	24	N.S.
Prednisone (25)	10.2	12.1	2.0	2.0	24	"
Morphine (25)	15.8	15.7	1.1	1.1	24	"
Aminopyrine (25)	29.7	17.7	1.4	1.4	30	<<.001

* $\bar{x}$ = mean; S.E. = stand. error; N = total No. of determinations divided equally among test and control groups; P values obtained by Student's "t" test; N.S. = not significant at $p = 0.05$.

† Day to day variation was seen in brain barbiturate levels, but were not significant statistically.

not elicit this effect.

The results summarized in Table II indicate that mice receiving sodium pentobarbital pretreated with phenylbutazone or aminopyrine show significantly increased brain barbiturate levels while those animals receiving prednisone, morphine or acetylsalicylic acid do not.

Discussion. One is tempted to speculate that those compounds producing prolongation of sleeping time and increased brain barbiturate levels may act by increasing permeability of the "blood-brain barrier," as apparently does physostigmine(3). However, it should also be considered that the phenylbutazone and aminopyrine effects may be due to inhibition of barbiturate metabolism as has been reported for many compounds(4). Our experiments throw no light on the mechanism of the effect which has been described. It would appear that the clinical impression that increased duration of hypnosis occurs with these combinations may be valid. It likewise appears that this effect is not related to anti-inflammatory action or analgesic potency.

Summary. The effect of acetylsalicylic acid, aminopyrine, morphine, phenylbutazone and prednisone on duration of hypnosis and brain barbiturate concentrations in pentobarbital anesthesia in mice has been determined. Among these agents, only aminopyrine and phenylbutazone significantly increased the brain barbiturate concentration and prolonged pentobarbital hypnosis. The other agents were without significant effect on these parameters.

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1. Finney, D. J., *Statistical Method in Biological Assay*, Hafner Publishing Co., N. Y., 1952.

2. Brodie, B. B., Burns, J. J., Mark, L. C., Lief, P. A., Bernstein, E., Papper, E. M., *J. Pharmacol. Exp. Therap.*, 1953, v109, 26.

3. Greig, N. E., Mayberry, T. C., *ibid.*, 1951, v102, 1.

4. Brodie, B. F., *J. Pharm. Pharmacol.*, 1956, v8, 1.

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